

Model Reduction and Analysis of Robustness for the Wnt/ β -Catenin Signal Transduction Pathway

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Abstract

We present a framework for model reduction of signal transduction networks. The methods are explained by considering a recent model for Wnt/ β -catenin signalling which plays an important regulatory role in cell development and oncogenesis. The procedure results in a reduction of system variables and parameters while maintaining the ability of the model to describe experimental data and to predict the in-vivo behaviour of the pathway. Using metabolic control analysis we quantified the response of the pathway towards random fluctuations of model parameters. This allows to characterise the robustness of the pathway against perturbations in stimulated and unstimulated states. We show that robustness depends on structural as well as kinetic properties of the pathway.

Keywords: signal transduction, Wnt, model reduction, control coefficient, robustness, time scale

1 Introduction

Mathematical modelling of signal transduction is becoming an important tool for elucidating the relation between structure and function of signalling networks [1, 2, 4]. It can be used to simulate experimental data, to predict dynamic phenomena not yet measured, and to identify key steps having a major effect on the function of the pathways. Generally, mathematical modelling starts with constituting a reaction scheme of the pathway or a special part of it. The scheme is then translated into a system of differential equations for the variables which are mostly the concentrations of the pathway compounds. The differential equations depend on kinetic constants which are often not accessible to experimental determination and must therefore be estimated by fitting the model to experimental data. In the present work we focus on methods for simplifying mathematical models of signal transduction pathways by reduction of the number of essential parameters and dynamic variables. The procedure is mainly based on time scale separation of the processes involved and gives rise to simple algebraic constraints as conservation equations and quasi-equilibrium conditions, replacing some of the original differential equations. We demonstrate the procedure of model reduction by considering a mathematical description of the Wnt/ β -catenin pathway which was developed recently [5]. Moreover, we enhance methods of the metabolic control analysis [3] to be applicable to signal transduction and to characterize the robustness of a pathway against parameter perturbations.

2 Results

2.1 Biological Background and Kinetic Description

The Wnt/ β -catenin signal transduction pathway is an essential part of the early development in eukaryotic organisms [10] and plays a key role in oncogenesis [6, 8]. The pathway controls the concentration of β -catenin in cooperation with proteasomal protein degradation. Upon stimulation of a membrane

receptor (Frizzled) by the ligand Wnt, β -catenin is stabilized and translocated into the nucleus where it activates the expression of target genes of the LEF/TCF group of transcription factors.

Our model is based on the reaction scheme depicted in Figure 1. Central to the scheme is a so-called destruction complex containing the phosphorylated forms of the scaffolds APC and Axin, as well as the kinase GSK3. It binds β -catenin and catalyses its phosphorylation (steps 8 and 9). Phosphorylated β -catenin is released from the complex (step 10), ubiquitinated and targeted for degradation by the proteasome (step 11). The destruction complex is formed by sequential binding of its components (steps 6 and 7). Steps 4 and 5 represent phosphorylation and dephosphorylation of the two scaffolds by GSK3 and a phosphatase, respectively. In the presence of a Wnt signal, the signalling protein Dishevelled is activated (step 1) leading to an inhibition of GSK3, probably by its release from the destruction complex (step 3). In turn, phosphorylation and degradation of β -catenin is impaired leading to its accumulation. The model also takes into account deactivation of Dsh (step 2), the syntheses of all proteins (steps 12, 14, 18, 20, 22, 24), their degradations (steps 13, 15, 19, 21, 23, 25), as well as the reversible binding of β -catenin to the transcription factor TCF (step 16) and to APC (step 17).

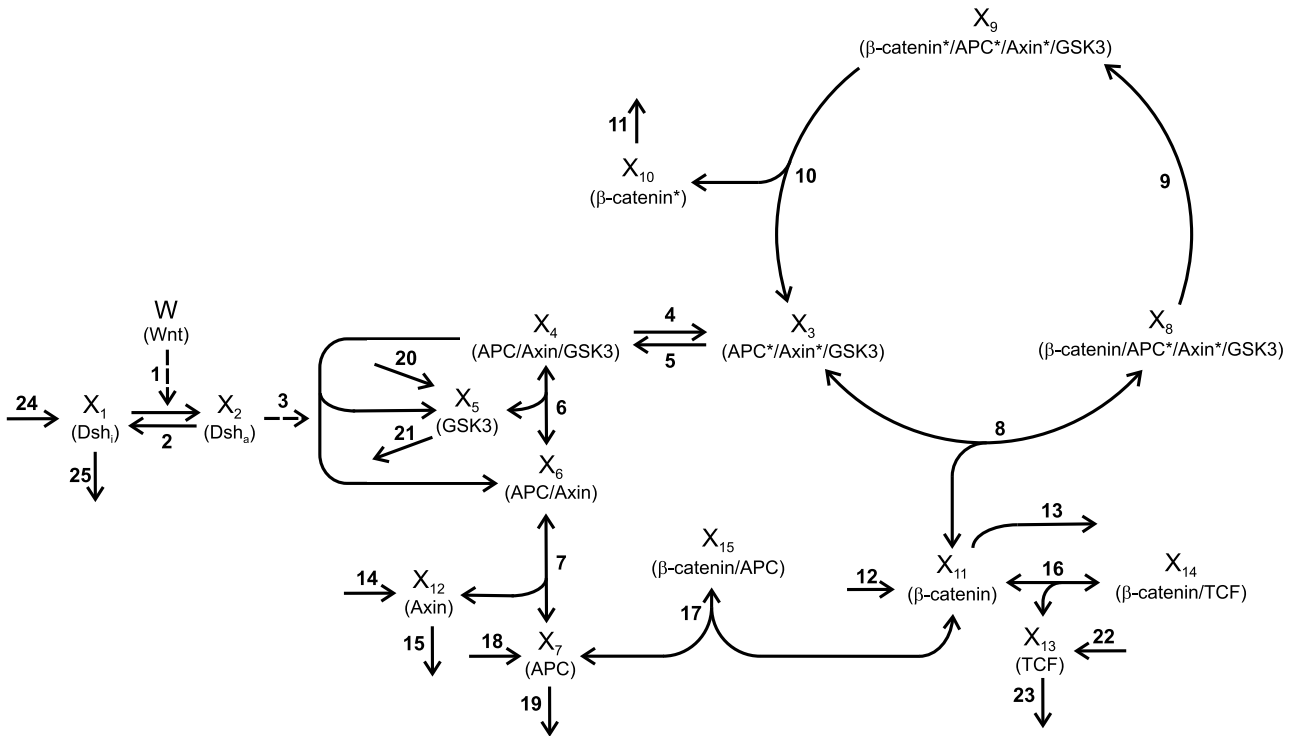


Figure 1: Reaction scheme for a model of the Wnt pathway. The reaction steps are numbered (1 to 25). For all compounds biochemical notations (names in brackets) and mathematical abbreviations are used (X_1 to X_{15}). Phosphorylated components are marked by an asterisk. Broken arrows characterize the activation of Dsh by Wnt (step 1) and the Dsh-mediated release of GSK3 (step 3). Double-headed arrows represent reversible binding reactions. For other details see text.

The mathematical model is based on a system of 15 ordinary differential equations (Table 1), governing the temporal changes of the concentrations of proteins and protein complexes in the Wnt pathway. Protein syntheses are characterized by constant rates, and the other processes are described by linear and bilinear rate equations. The complete set of 25 rate equations with a total of 31 kinetic parameters is given in Table 2.

2.2 Model Reduction for the Wnt Pathway

The model is simplified by limiting the analysis to processes proceeding on a timescale of hours. Some processes are very fast allowing for the application of quasi-steady-state approximations or rapid equilibrium approximations. Very slow processes can be neglected leading to the occurrence of conservation relations [3]. For both cases some of the differential equations are replaced by algebraic equations which can be used to express several dependent variables as functions of independent variables. The latter variables are determined as solutions of a smaller set of differential equation not containing the dependent variables.

Table 1: System of differential equations for the scheme in Figure 1.

| component | equation | component | equation |
|-----------|-----------------------------------------------------|-----------|-----------------------------------------------------------------|
| 1 | $\frac{dX_1}{dt} = -V_1 + V_2 + V_{24} - V_{25}$ | 9 | $\frac{dX_9}{dt} = V_9 - V_{10}$ |
| 2 | $\frac{dX_2}{dt} = V_1 - V_2$ | 10 | $\frac{dX_{10}}{dt} = V_{10} - V_{11}$ |
| 3 | $\frac{dX_3}{dt} = V_4 - V_5 - V_8 + V_{10}$ | 11 | $\frac{dX_{11}}{dt} = -V_8 + V_{12} - V_{13} - V_{16} - V_{17}$ |
| 4 | $\frac{dX_4}{dt} = -V_3 - V_4 + V_5 + V_6$ | 12 | $\frac{dX_{12}}{dt} = -V_7 + V_{14} - V_{15}$ |
| 5 | $\frac{dX_5}{dt} = V_3 - V_6 + V_{20} - V_{21}$ | 13 | $\frac{dX_{13}}{dt} = -V_{16} + V_{22} - V_{23}$ |
| 6 | $\frac{dX_6}{dt} = V_3 - V_6 + V_7$ | 14 | $\frac{dX_{14}}{dt} = V_{16}$ |
| 7 | $\frac{dX_7}{dt} = -V_7 - V_{17} + V_{18} - V_{19}$ | 15 | $\frac{dX_{15}}{dt} = V_{17}$ |
| 8 | $\frac{dX_8}{dt} = V_8 - V_9$ | | |

Conservation Equations

Our reaction scheme includes synthesis and degradation of all proteins. However, experimental examination of protein degradation reveals that there is no detectable degradation of APC, TCF, Dsh and GSK3 in several hours whereas β -catenin and Axin are rapidly degraded with half-lives of 60 min and 6 min, respectively [5]. Therefore, we neglect syntheses and degradations of APC, TCF, Dsh and GSK3 (rates V_{18} to V_{25}) which implies four conservation relations [7] resulting from linear dependencies of the rows of the stoichiometric matrix N depicted in Figure 2

$$X_1 + X_2 = \text{const.} \quad (1)$$

$$X_3 + X_4 + X_5 + X_8 + X_9 = \text{const.} \quad (2)$$

$$X_3 + X_4 + X_6 + X_7 + X_8 + X_9 + X_{15} = \text{const.} \quad (3)$$

$$X_{13} + X_{14} = \text{const.} \quad (4)$$

The four conserved quantities in Eqs. (1) to (4) correspond to the total concentrations Dsh^0 , $GSK3^0$, APC^0 , and TCF^0 , respectively. We use the conservation equations (2) and (3) for GSK3 and APC in the more simplified forms

$$X_5 = GSK3^0 \tag{5a}$$

$$X_7 + X_{15} = APC^0, \tag{5b}$$

which are justified since all other terms on the left-hand side of the Eqs. (2) or (3) represent the concentrations of the complexes containing also Axin which occurs in extremely low concentration (see [5, 11]) compared to GSK3 and APC.

Table 2: Reaction rates for the model.

| step | rate equation | process |
|------|---------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| 1 | $V_1 = k_1 W X_1$ | activation of Dsh by Wnt |
| 2 | $V_2 = k_2 X_2$ | deactivation of Dsh |
| 3 | $V_3 = k_3 X_2 X_4$ | dissociation of GSK3 from the destruction complex |
| 4 | $V_4 = k_4 X_4$ | phosphorylation of Axin and APC |
| 5 | $V_5 = k_5 X_3$ | dephosphorylation of Axin and APC |
| 6 | $V_6 = k_{+6} X_5 X_6 - k_{-6} X_4$ | binding of GSK3 to the (APC/Axin) complex and dissociation of GSK3 from the destruction complex |
| 7 | $V_7 = k_{+7} X_7 X_{12} - k_{-7} X_6$ | binding of APC to Axin and dissociation of APC from the (APC/Axin) complex |
| 8 | $V_8 = k_{+8} X_3 X_{11} - k_{-8} X_8$ | binding of β -catenin to the destruction complex and dissociation of β -catenin from the destruction complex |
| 9 | $V_9 = k_9 X_8$ | phosphorylation of β -catenin |
| 10 | $V_{10} = k_{10} X_9$ | dissociation of phosphorylated β -catenin |
| 11 | $V_{11} = k_{11} X_{10}$ | degradation of phosphorylated β -catenin |
| 12 | $V_{12} = \bullet$ | synthesis of β -catenin |
| 13 | $V_{13} = k_{13} X_{11}$ | degradation of β -catenin |
| 14 | $V_{14} = \bullet$ | synthesis of Axin |
| 15 | $V_{15} = k_{15} X_{12}$ | degradation of Axin |
| 16 | $V_{16} = k_{+16} X_{13} X_{14} - k_{-16} X_{14}$ | binding of TCF to β -catenin and dissociation of TCF from (β -catenin/TCF) |
| 17 | $V_{17} = k_{+17} X_7 X_{11} - k_{-17} X_{15}$ | binding of APC to β -catenin and dissociation of APC from (β -catenin/APC) |
| 18 | $V_{18} = \bullet$ | synthesis of APC |
| 19 | $V_{19} = k_{19} X_7$ | degradation of APC |
| 20 | $V_{20} = \bullet$ | synthesis of GSK3 |
| 21 | $V_{21} = k_{21} X_5$ | degradation of GSK3 |
| 22 | $V_{22} = \bullet$ | synthesis of TCF |
| 23 | $V_{23} = k_{23} X_{13}$ | degradation of TCF |
| 24 | $V_{24} = \bullet$ | synthesis of Dsh |
| 25 | $V_{25} = k_{25} X_1$ | degradation of Dsh |

Equilibrium Conditions

Among the five reversible binding steps (steps 6 to 8, 16, and 17) all except step 6 are assumed to be very fast compared to the other processes. Accordingly, the model describes the binding of

The binding equilibrium between β -catenin and APC, Eq. (6d), and the conservation equation for APC, Eq.(5b) allow to express X_7 and X_{15} as functions of X_{11} :

$$X_7 = \frac{K_{17}APC^0}{K_{17} + X_{11}} \quad (8a)$$

$$X_{15} = \frac{X_{11}APC^0}{K_{17} + X_{11}}. \quad (8b)$$

Similarly, Eq.(6c) characterizing the equilibrium between β -catenin and TCF is used to write X_{13} and X_{14} as functions of X_{11} :

$$X_{13} = \frac{K_{16}TCF^0}{K_{16} + X_{11}} \quad (9a)$$

$$X_{14} = \frac{X_{11}TCF^0}{K_{16} + X_{11}}. \quad (9b)$$

The concentration of the (β -catenin/APC*/Axin*/GSK3) complex follows directly from the Eq.(6b) by rewriting X_8 as a function of X_3 and X_{11}

$$X_8 = \frac{X_3X_{11}}{K_8}. \quad (10)$$

Taking into account Eq.(8a) for X_7 and using Eq.(6a) the concentration of the (APC/Axin) complex, X_6 , can be expressed as function of X_{11} and X_{12} :

$$X_6 = \frac{K_{17}X_{12}APC^0}{K_7(K_{17} + X_{11})}. \quad (11)$$

Differential Equations of the Independent Variables

We first consider the differential equations for X_2 , X_4 , X_9 , and X_{10} which are not affected by the rapid equilibrium approximations. These equations can be easily rewritten by introducing the rate equations given in Table 2 and by eliminating the dependent variables using the Eqs. (5a) and (7) to (11). This leads to

$$\frac{dX_2}{dt} = k_1W(Dsh^0 - X_2) - k_2X_2, \quad (12)$$

$$\frac{dX_4}{dt} = k_5X_3 + k_6\frac{K_{17}X_{12}APC^0GSK3^0}{K_7(K_{17} + X_{11})} - (k_3X_2 + k_4 + k_{-6})X_4, \quad (13)$$

$$\frac{dX_9}{dt} = k_9X_8 - k_{10}X_9 = \frac{k_9X_3X_{11}}{K_8} - k_{10}X_9, \quad (14)$$

$$\frac{dX_{10}}{dt} = k_{10}X_9 - k_{11}X_{10}. \quad (15)$$

The differential equations of the remaining independent variables X_3 , X_{11} , and X_{12} contain the rate equations affected by rapid binding and dissociation processes, V_7 , V_8 , V_{16} , and V_{17} . To derive differential equations for the variations on the slow time-scale, these reaction rates must be eliminated which can be achieved by appropriate linear combination of the original differential equations (for details of the method cf. [3]). It is easy to see that for the present scheme of the Wnt pathway, the time-dependent variations of the three pool variables $P_1 = X_6 + X_{12}$, $P_2 = X_3 + X_8$, and $P_3 = X_8 + X_{11} + X_{14} + X_{15}$ depend solely on the rates of slow processes. For the first pool variable the differential equation reads

$$\frac{dP_1}{dt} = \frac{d(X_6 + X_{12})}{dt} = V_3 - V_6 + V_{14} - V_{15}. \quad (16)$$

The left-hand side as well as V_6 on the right-hand side of this equation still contain the dependent variable X_6 which must be eliminated. This can be done on the basis of Eq. (11), which describes X_6 as a function of X_{11} , and X_{12} . Accordingly, its time variation can be expressed as follows

$$\frac{dX_6}{dt} = \frac{\partial X_6}{\partial X_{11}} \frac{dX_{11}}{dt} + \frac{\partial X_6}{\partial X_{12}} \frac{dX_{12}}{dt}. \quad (17)$$

Calculating the derivatives $\partial X_6/\partial X_{11}$ and $\partial X_6/\partial X_{12}$ from Eq. (11) and introducing on the right hand side of Eq. (16) the rate equations from Table 2 yields

$$\begin{aligned} \frac{dP_1}{dt} &= \frac{dX_{12}}{dt} \left(1 + \frac{APC^0 K_{17}}{K_7(K_{17} + X_{11})} \right) - \frac{dX_{11}}{dt} \frac{APC^0 K_{17} X_{12}}{K_7(K_{17} + X_{11})^2} \\ &= k_3 X_2 X_4 - k_6 \frac{GSK3^0 APC^0 K_{17} X_{12}}{K_7(K_{17} + X_{11})} + k_{-6} X_4 + V_{14} - k_{15} X_{12}, \end{aligned} \quad (18)$$

which depends solely on the independent variables. In an analogous way, we obtain

$$\begin{aligned} \frac{dP_2}{dt} &= \left(1 + \frac{X_{11}}{K_8} \right) \frac{dX_3}{dt} + \frac{X_3}{K_8} \frac{dX_{11}}{dt} = k_4 X_4 - k_5 X_3 - \frac{k_9 X_3 X_{11}}{K_8} + k_{10} X_9 \text{ and} \\ \frac{dP_3}{dt} &= \frac{dX_{11}}{dt} \left(1 + \frac{X_3}{X_8} + \frac{TCF^0 K_{16}}{(K_{16} + X_{11})^2} + \frac{APC^0 K_{17}}{(K_{17} + X_{11})^2} \right) + \frac{X_{11}}{K_8} \frac{dX_3}{dt} = V_{12} - \left(\frac{k_9 X_3}{K_8} + k_{13} \right) X_{11}. \end{aligned} \quad (19)$$

(20)

In summary, the original system of 15 differential equations involving 31 parameters (Table 1) is reduced to a system of 7 differential equations (Eqs. (12) to (15) and Eqs. (18) to (20)) and 8 simple algebraic equations (Eqs. (5a), (7) to (11)) with only 19 parameters.

Application of the Model

We have shown [5] that the reduced model described above is able to reproduce experimental results, such as time courses for β -catenin degradation as observed in *Xenopus* oocyte extracts. In agreement with the experiments, our model revealed that the very low concentration of Axin is crucial for the control of the β -catenin level. Moreover, we made predictions for the in-vivo behaviour of the Wnt pathway under transient and permanent stimulation and identified the Axin turnover as an essential factor for transient β -catenin signalling. We proposed that Axin degradation may be a potential drug target to counteract the effect of some APC mutations which result in decreased APC levels. Since both APC and Axin bind each other to form the β -catenin destruction complex a decrease of APC leads to a reduction of this complex and to higher β -catenin levels. Since the concentration of APC is much higher than the concentration of Axin, an increase of Axin is able to compensate the decreased levels of the destruction complex. In extension to the previous work we present here two simulations showing how the β -catenin signal depends (A) on the duration of a transient stimulation of the pathway by its ligand Wnt, and (B) on the characteristic time τ_{PD} of the phosphorylation/dephosphorylation cycle of Axin and APC. The transient stimulus is simulated by $W(t) = \exp(-t/\tau_W)$, in which τ_W represents the characteristic time of stimulus decay.

Figure 3 shows that the duration and amplitude of the signal depends crucially on the kinetics of stimulation whereas the phosphorylation/dephosphorylation rate of Axin and APC has only a minor effect. A higher value of the stimulus time leads to prolonged and increased signalling. For a detailed discussion of the effects of signal amplification and signal duration see [2].

2.3 Analysis of Robustness

Complex reaction systems like the Wnt model contain many parameters which affect the components of the pathway to different extents. Methods of metabolic control analysis (MCA) can be used to identify

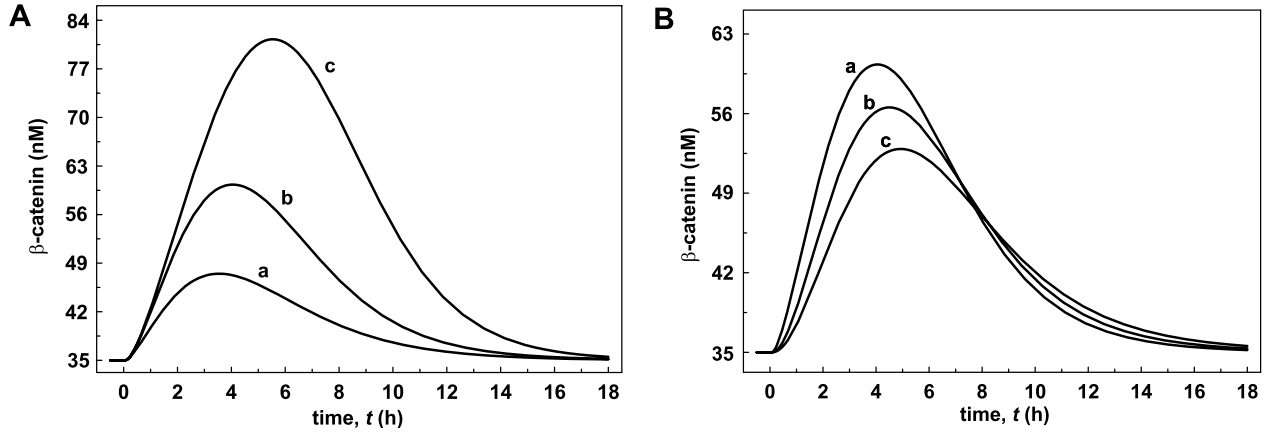


Figure 3: Time dependent variation of the β -catenin signal upon transient stimulation of the Wnt pathway (A) with a stimulus time of (a) $\tau_W = 5$ min; (b) $\tau_W = 20$ min, (c) $\tau_W = 60$ min, and (B) with a characteristic time of the phosphorylation/dephosphorylation cycle of axin and APC of (a) $\tau_{PD} = 2.5$ min, (b) $\tau_{PD} = 12.5$ min, and (c) $\tau_{PD} = 25$ min. All simulations were performed numerically using Mathematica[®] 5, Wolfram Research, Champaign, IL. The Mathematica notebook file is freely available via our website [11].

key parameters having strongest effects on the behaviour of the network [3]. Here we extend this concept to characterize the robustness of signalling networks against variations of kinetic parameters. We restrict our analysis to steady states although it can be easily extended to transient states. For quantifying the response of a concentration X_i towards changes of the rates V_j of individual reactions we use the control coefficients

$$C_j^i = \frac{V_j}{X_i} \frac{\partial X_i / \partial k_j}{\partial V_j / \partial k_j} \quad (21)$$

The various coefficients constitute a control matrix \mathbf{C} relating the relative changes of the system variables $x_i = \Delta X_i / X_i$ ($1 \leq i \leq n$) to the perturbations $v_j = \Delta V_j / V_j$ ($1 \leq j \leq r$)

$$\mathbf{x} = \mathbf{C}\mathbf{v} \text{ with } \mathbf{x} = \begin{pmatrix} \Delta X_1 / X_1 \\ \vdots \\ \Delta X_n / X_n \end{pmatrix}, \mathbf{C} = \begin{pmatrix} C_1^1 & \cdots & C_r^1 \\ \vdots & \ddots & \vdots \\ C_1^n & \cdots & C_r^n \end{pmatrix}, \text{ and } \mathbf{v} = \begin{pmatrix} \Delta V_1 / V_1 \\ \vdots \\ \Delta V_r / V_r \end{pmatrix}. \quad (22)$$

(since the rate constants enter the rate equations in a linear way one gets $\Delta V_j / V_j = \Delta k_j / k_j$). Assuming independent and random fluctuations of the rate constants the mean values $\langle \Delta V_j / V_j \rangle$ will be zero which implies $\langle \Delta X_i / X_i \rangle = 0$. In contrast, the variances and covariances of \mathbf{x} will generally not vanish. They are the diagonal elements and non-diagonal elements of the covariance matrix

$$\langle \mathbf{x}\mathbf{x}^T \rangle = \langle (\mathbf{C}\mathbf{v})(\mathbf{C}\mathbf{v})^T \rangle = \langle \mathbf{C}(\mathbf{v}\mathbf{v}^T)\mathbf{C}^T \rangle \quad (23)$$

Since the reaction rates fluctuate independently the non-diagonal elements of $\mathbf{v}\mathbf{v}^T$ do not contribute to $\langle \mathbf{x}\mathbf{x}^T \rangle$. For simplicity we assume that $v^2 = \langle v_j^2 \rangle$ for all j . In this way Eq. (23) rearranges into

$$\frac{\langle \mathbf{x}\mathbf{x}^T \rangle}{v^2} = \mathbf{C}\mathbf{C}^T, \text{ where} \quad (24)$$

$$\mathbf{C}\mathbf{C}^T = \begin{pmatrix} (C_1^1)^2 + \cdots + (C_r^1)^2 & \cdots & C_1^1 C_1^n + \cdots + C_r^1 C_r^n \\ \vdots & \ddots & \vdots \\ C_1^1 C_1^n + \cdots + C_r^1 C_r^n & \cdots & (C_1^n)^2 + \cdots + (C_r^n)^2 \end{pmatrix}. \quad (25)$$

The diagonal elements of $\mathbf{C}\mathbf{C}^T$ are the variances of the individual x_i normalised to the variance v^2 of the relative changes in the reaction rates. The average effect of the perturbation of a single parameter on a particular concentration can therefore be described by

$$\sigma_i^2 = \frac{1}{r} \sum_{j=1}^r (C_j^i)^2. \quad (26)$$

Similarly to the individual variances, one can define an overall variance which is given by the sum of the individual variances of \mathbf{x} , that is $\langle \mathbf{x}^2 \rangle = v^2 \text{Tr}(\mathbf{C}\mathbf{C}^T)$. Analogously to Eq. (26), we define the average effect of the perturbation of a single parameter on a single concentration by

$$\sigma^2 = \frac{1}{n \cdot r} \text{Tr}(\mathbf{C}\mathbf{C}^T). \quad (27)$$

A low value of σ^2 characterises a high robustness of the system against parameter perturbations while a high value of σ^2 implies a low robustness. Normalisation of the variances with respect to the number n of compounds and the number r of reactions (Eqs. (26) and (27)) allows for comparing the robustness of pathways of different size. Moreover, the individual compound can be classified to be robust or sensitive by comparing for any i the values of σ_i^2 with their average σ^2 .

This concept of robustness is now applied to the model of the Wnt pathway. Two different stationary states are considered, the unstimulated reference state, and the standard stimulated state characterized by $W = 0$ and $W = 1$, respectively. The corresponding concentrations of proteins and protein complexes are listed in Table 3 in the first columns for each state. The major effect of pathway stimulation is the increase of the concentration of dephosphorylated β -catenin, in agreement with experimental data (for recent data see [9]). Another important effect is the decrease of the concentration of the destruction complex. The second columns for each state contain the individual relative variances σ_i^2 . For determining these values by Eq. (26) we first calculated a full set of control coefficients according to Eq. (21). The average relative variances (cf. Eq. (27)) for both states are shown in the last row of Table 3. The values for the individual variances above-average are written in bold face. In the unstimulated state the group of sensitive components includes unphosphorylated β -catenin in its free form and in its binary complex with APC as well as the two forms of the destruction complex. Interestingly, the values of σ_i^2 for all free proteins except for unphosphorylated β -catenin are below-average. The most remarkable effect upon the transition from the reference state to the stimulated state is the decrease of the variance of the (β -catenin/TCF) complex by more than a factor of ten. All other σ_i^2 values, except for free TCF, display only minor changes. In view of the fact that signal transmission is eventually mediated by the (β -catenin/TCF)-complex, this result suggests that the signalling output in the stimulated state is very robust against parameter fluctuations. However, in the unstimulated state, the concentration of the (β -catenin/TCF) complex is much more sensitive. This indicates that parameter fluctuations or individual parameter changes may result in an accumulation of the (β -catenin/TCF) complex. This could mimic an activation of the pathway even in the absence of stimulation leading to inappropriate consequences for the cell fate.

3 Discussion

We present a procedure of model reduction for signal transduction pathways exemplified by a model for the Wnt/ β -catenin pathway published recently [5]. The procedure is mainly based on time scale separation leading (a) to conservation relations for components characterised by a slow protein turnover and (b) to quasi-equilibrium relations for fast binding processes. In this way, the number of independent model variables is reduced. As demonstrated here, the reduced system is able to describe the behaviour of the Wnt pathway within a specific timescale. Since protein synthesis and degradation as

Table 3: Concentrations and variances calculated for two stationary states of the Wnt pathway.

| Component | i | $W = 0$ | | $W = 1$ | |
|-----------------------------------------|-----|---------------------|--------------------|---------------------|----------------------|
| | | X_i (nM) | σ_i^2 | X_i (nM) | σ_i^2 |
| Dsh _i | 1 | - | - | 9,09 | 0,075 |
| Dsh _a | 2 | - | - | 90,9 | 0,001 |
| (APC*/ Axin*/ GSK3) | 3 | 0,010 | 0,438 | 0,002 | 0,441 |
| (APC/Axin/GSK3) | 4 | 0,005 | 0,329 | 0,001 | 0,329 |
| GSK3 | 5 | 50 | (1 ⁻⁹) | 50 | (10 ⁻¹⁰) |
| (APC/Axin) | 6 | 0,001 | 0,219 | 0,001 | 0,232 |
| APC | 7 | 97,9 | (10 ⁴) | 88,7 | 0,009 |
| (β -catenin/ APC*/ Axin*/ GSK3) | 8 | 0,002 | 0,104 | 0,002 | 0,086 |
| (β -catenin*/APC*/Axin*/GSK3) | 9 | 0,002 | 0,105 | 0,002 | 0,095 |
| β -catenin* | 10 | 1 | 0,105 | 0,921 | 0,095 |
| β -catenin | 11 | 25,1 | 0,639 | 153 | 0,557 |
| Axin | 12 | (10 ⁻⁴) | 0,105 | (10 ⁻⁴) | 0,091 |
| TCF | 13 | 8,17 | 0,155 | 2,46 | 0,453 |
| (β -catenin/TCF) | 14 | 6,84 | 0,221 | 12,5 | 0,017 |
| (β -catenin/APC) | 15 | 2,05 | 0,719 | 11,3 | 0,526 |
| σ^2 | | | 0,242 | | 0,200 |

well as formation of protein complexes are ubiquitous in signal transduction the procedure should be also applicable to other pathways. At first view the reduction of the number of differential equations does not seem to gain advantages since both the original as well as the reduced system might be solved by the same methods. However, with respect to the analysis of signalling systems there are at least three relevant advantages of this reduction procedure. (a) The number of system parameters is also considerably reduced which is very useful since parameters are often unknown or experimentally hardly accessible. (b) The reduced system only describes effects on the biologically relevant timescale and, therefore, (c) contains less stiff differential equations which can be solved faster and easier.

Moreover, we introduce in this paper a concept for analysing the robustness of signalling pathways towards parameter fluctuations. We define variances characterizing the effects of random parameter perturbations on the concentrations of individual components as well as the corresponding averaged effects on the whole system. The analysis is performed in terms of control coefficients depending not only on the structure of a given pathway but also on the kinetic properties of its individual reactions. In future work analysis of robustness can be extended to properties of signalling pathways characterizing transient states such as signal propagation time, signal duration, as well as signalling amplitude [2].

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